Date: 7/30/13 People in lab: Emily Puleo, Kelsey Crossen

Title: Preparation of P. aeruginosa

Start Time: 3:00 PM

Purpose: To make a stock of P. aeruginosa for future lab work.

Protocol: Inoculated LB broth with P. aeruginosa from plated bacteria, no antibiotic. Inoculated another tube of LB broth with liquid P. aeruginosa culture, 10ml into 10ml.

Left both cultures in 37C incubator overnight.

Spun down 1ml cultures of P. aeruginosa, discarded supernatant, and re-suspended in 1ml Milli-Q water.

Placed cell solutions in 1.5ml Epi tubes and placed in -20C freezer.

Products:

Sample Label	Description	Source Label	Quantity
P. aeruginosa 7/30	P. aeruginosa culture	P. aeruginosa	2
P. aeruginosa 7/30	P. aeruginosa culture, frozen	P. aeruginosa	10

Stop Time: 5:00 PM

Next: Amplify DNA segments from prepared P. aeruginosa samples.

Date: 7/31/13 People in lab: Emily Puleo, Kelsey Crossen

Title: PCR of norCB, dilution of norCB primers

Start Time: 4:57 PM

Purpose: PCR norCB from P. aeruginosa, adding iGEM prefix and suffix; to prepare primers for future lab work

Protocol: LTM ed. 2 pg. 48 **Exceptions:** 1. Less than 0.1 uM final primer concentration.

Products:

Sample Label	Description	Source Label	Quantity
7/31/13 A norCB 1	Amplified norCB, with prefix/suffix, 7.5uL template	P. aeruginosa 7/30	1
7/31/13 A norCB 2	Amplified norCB, with prefix/suffix, 5.63uL template	P. aeruginosa 7/30	1
7/31/13 A norCB 3	Amplified norCB, with prefix/suffix, 3.75uL template	P. aeruginosa 7/30	1
7/31/13 norCB F	Forward primer for norCB, 0.73 uM	norCB Forward Primer	1
7/31/13 norCB R	Reverse primer for norCB, 0.87 uM	norCB Reverse Primer	1

Results: Sample 3 had too little product and was discarded.

Notes: Different amounts of template used to find optimum amount needed.

Forward primer: (24.2*10^-9)/(0.33*10^-3) mol/L = 73 uM, (73uM)(10uL)=(0.73uM)V2, V2 = 1000uL

Reverse primer: (28.7*10^-9)/(0.33*10^-3) mol/L = 87 uM, (87uM)(10uL)=(0.87uM)V2, V2 = 1000uL

Used Dr. Shannon's thermocycler, copied "igem_pcr" program

Stop Time: 9:00 PM

Next: Run samples on a gel, extract the stronger band from between them, sequence the result.